

## INTRODUCTION

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A unique feature of the avian spinal cord is the presence of a wedge shaped structure called the *glycogen body*, situated mid-dorsally in the lumbosacral region of the spinal cord (Terni, 1924; Watterson, 1949; DeGennaro, 1982; Fig. 1 and 2). In addition to glycogen body, there are also present a series of comparatively very small and therefore less conspicuous *accessory lobes of Lachi* (Lachi, 1889), which lie along the proximal portion of ventral motor routes of spinal nerves 19 to 29 (Lachi, 1889). Both glycogen body and lobes of Lachi (Fig. 4) are made up of astroglial cells which possess large quantities of glycogen (DeGennaro and Benzo, 1978). The glycogen body comprising of astroglial cells makes its appearance in the embryo of chick (*Gallus gallus*) around 7 days of incubation. Subsequently, the cells proliferate and accumulate enormous quantities of glycogen in its cells (Doyle and Watterson, 1949; Watterson, 1949, 1951, 1952a & b; DeGennaro, 1959), and persist throughout the life of the bird.

It was Duval (1877) who made the first notable attempt to unravel the nature of glycogen body tissue. Since then several researchers have undertaken work with a view to elucidate its structural and functional significance in

Fig. 1 Photograph showing dorsal view of glycogen body in the lumbosacral nerve cord of 3 week old chicken. Note that glycogen body is placed between cruralis (upper arrow) and sciatic nerves (lower arrow) of the lumbosacral plexi.

(From DeGennaro, 1982)

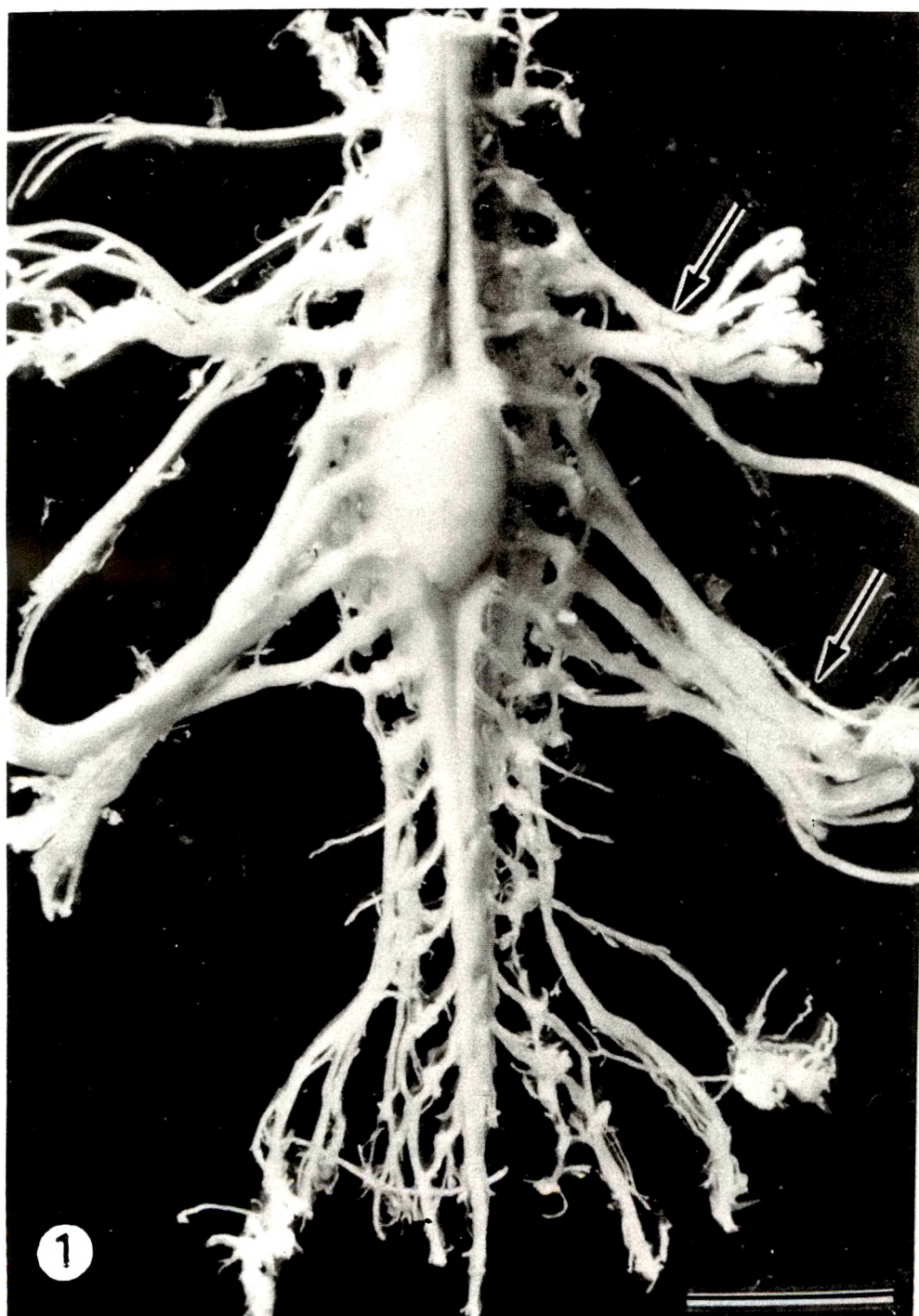


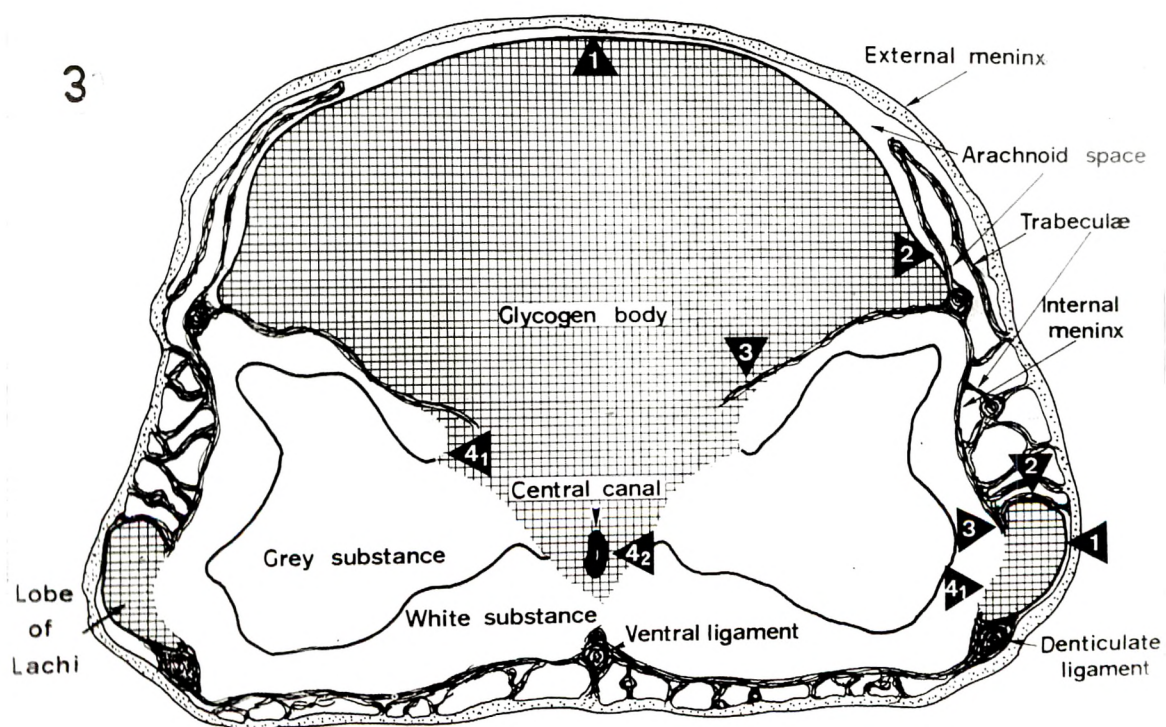
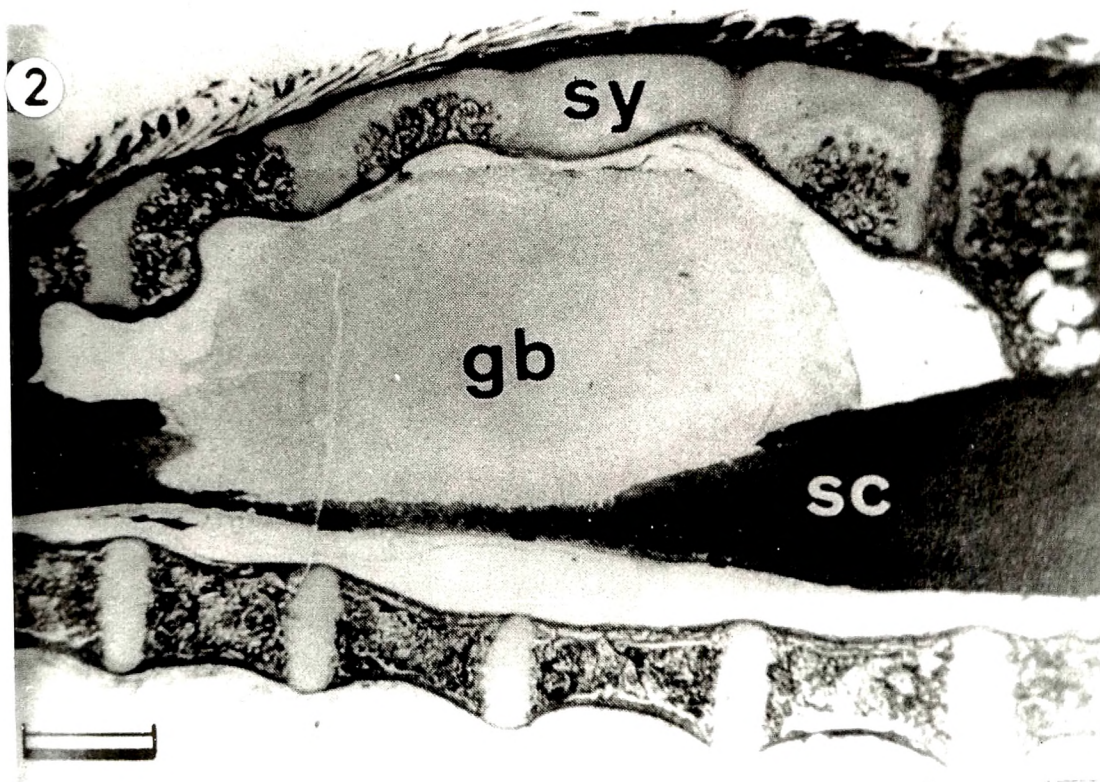
Fig. 2 Lateral view of lumbosacral spinal cord (sc) showing relationship with glycogen body (gb) and synsacrum (sy).

(From Azcoitia *et al.*, 1987)

Fig. 3 Diagrammatic sketch of transverse section of glycogen body. Morphological relationship with lobes of Lachi, nervous parenchyma and meninges is represented. Note that the internal meninx (pia) is disrupted dividing glycogen body into dorsal and ventral parts. 1- external meninx, 2- arachnoid trabeculae, 3- internal meninx, 4<sub>1</sub>- nervous parenchyma and 4<sub>2</sub>- ependyma.

(From Azcoitia *et al.*, 1987)





the birds. Imof (1905) described the presence of this structure in more than 30 species of birds (both terrestrial as well as aquatic in habitat). It was Terni (1924, 1926) who employing<sup>ed</sup> histochemical technique<sup>t</sup> demonstrated<sup>the</sup> presence of glycogen in the cells of this tissue. Subsequently, Watterson (1949) confirmed the earlier work of Terni and coined the term *glycogen body*. First comprehensive review on glycogen body was written about a decade<sup>and half</sup> ago (DeGennaro, 1982). Since then researchers from very few laboratories have been engaged in continuing work on this tissue and accordingly very few reports have appeared. Following are the details of the same covered under different headings.

#### GROSS MORPHOLOGY:

In *Gallus gallus*, glycogen body is found in the spinal segments 26-29 (Watterson, 1949). As regards relationship of meninges with glycogen body there is a general agreement regarding the fact that the upper that is dorsal part of glycogen body is enveloped by pia cranially, laterally and caudally (Dickson and Millen, 1957) which means that glycogen body tissue is separated from neural tissue of spinal cord in the dorsal half of the spinal cord whereas the ventral part of the glycogen body

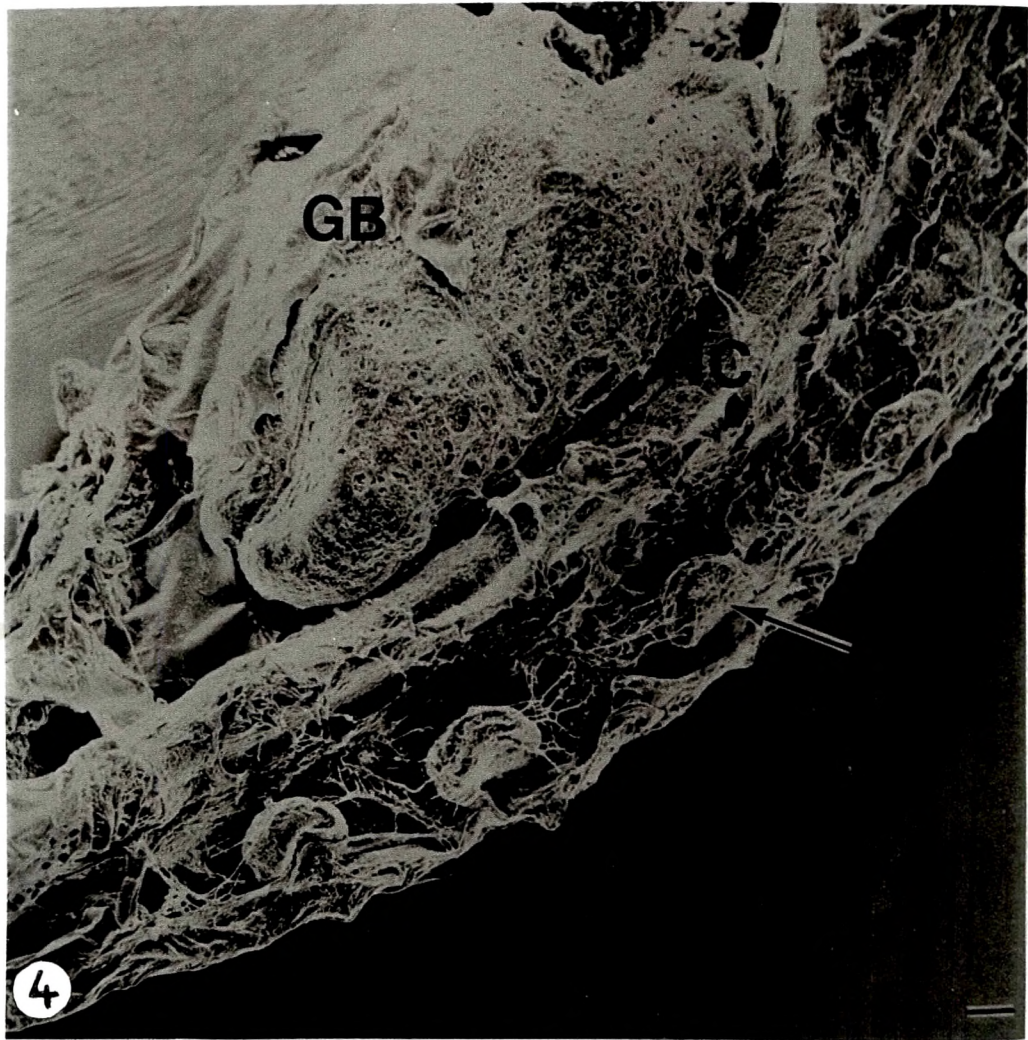
comprising of the lower part of the wedge shaped body that surrounds the central canal abuts the neural tissue directly, there being no pia in the lower region. However, the views regarding the nature of free dorsal surface of the glycogen body are contradictory. According to Dickson and Millen (1957) the dorsal surface is covered by pia. The spanish team comprising of Azcoitia *et al.*, (1987) on the basis of ultrastructural studies claim that growth of the glycogen body dorsally breaks the pia formed earlier so that after completion of development, glycogen body tissue projects into the subarachnoid space, there being no intervening pia (Azcoitia *et al.*, 1987; Fig. 3). These authors even suggest an analogy with the condition <sup>absence</sup> nonpossession in Foramen magnum which is characterized by nonpossession of pia.

Glycogen body is highly vascularised during early phases of incubation in chicken (Feeney and Watterson, 1946). Pessacq (1964) is of the view that the glycogen body shows presence of a network of portal blood vessels. According to Pessacq (1969), the vascular supply of glycogen body consists of one or two arteriolar trunks which enter from the dorsal face of the tissue, breaking up into capillaries that mainly lie scattered in the central zone with fewer numbers in the peripheral area. More recently

Fig. 4    Photograph showing lateral view of glycogen body (GB) and accessory lobes of lachi (arrow) as seen through scanning electron microscope in day old chick. NC- nerve cord.

(From DeGennaro, 1982)





Pessacq (1984) has <sup>observed</sup> ~~disclosed~~ the presence of nerve fibres in the glycogen body and found them to be widely distributed in the subependymal region.

#### IN-OVO DEVELOPMENT:

The development of glycogen body of the chicken has been the object of many investigations. Work pertaining to the origin of glycogen body has been diverse. Some researchers consider it to be a derivative of the meninges that invest the portion of spinal cord (Hansen-Prus, 1923; Ariens Kappers, 1924). However, the contention of these workers have been challenged by Watterson (1952a) who found the meningeal cells and the glycogen body cells to be different in their staining reactions to Best's Carmine. Further, staining of these cells with Ehrlich's haematoxylin revealed the nuclei of the meningeal cells to be small and elongated whereas the nuclei of glycogen body cells were large and more rounded. Based on these observations, Watterson (1952b) suggested the glycogen body to be neural in origin and his view is in <sup>conformity</sup> ~~unison~~ with <sup>those of</sup> early workers (Duval, 1877; Imof, 1905; Terni, 1924). Welsch and Wachtler (1969) and Lyser (1973) ~~are of the~~ <sup>ed</sup> opinion that the glycogen body may be a mass of specialized astroglia with a function to store glycogen in

its cells. Still others like Uehara and Ueshima (1982) think these cells to be derivative of the ventricular cells in the roof plate of neural tube.

The glycogen body makes its first distinct appearance as a paired primordia on either side of the roof plate of the embryonic nerve cord of chicken by about 7-8 days of incubation (Watterson, 1947, 1949; DeGennaro, 1959; Uehara and Ueshima, 1982). These primordia extend right upto the dorsal aspect of the central canal of the lumbosacral spinal cord (Watterson, 1949). Staining of the cells in the roof plate either with Best's Carmine (Watterson, 1949) or with per-iodic acid-Schiff's reagent using diastase as a control for glycogen (DeGennaro, 1959) has confirmed the material in the cells to be glycogen. The primordia of the glycogen body subsequently increase in mass. The paired primordia begin to fuse from the dorsal side of lumbosacral spinal cord by the 8<sup>th</sup> day of incubation (Uehara and Ueshima, 1982), gradually progressing dorsoventrally to finally fuse into a single mass by the 10<sup>th</sup> day of incubation in the chicken (Watterson, 1949; Uehara and Ueshima, 1982). Further, Uehara and Ueshima (1982) observed bilateral clusters of glycogen containing cells in the ventrolateral region of the lumbosacral cord, just below the central canal at stage 35 (Hamburger and Hamilton,

1951). They further observed that the cells subsequently increase in number and glycogen content, to finally abut with the cells on the other side. The two originally separate ventrolateral regions thus merge with each other and also become contiguous with the glycogen body. Therefore, Uehara and Ueshima (1982) consider them as the ventral paired primordia of the glycogen body and propose that the cells of the floor plate also contribute to the formation of the glycogen body.

#### **BIOCHEMICAL ASPECTS:**

→ Glycogen in the glycogen body constitutes 5-10% of total embryonic glycogen in the 14 day chick embryo (Watterson, 1949); Snedecor et al. (1963) have reported normal glycogen value of 25.7 mg% tissue wet weight in day old post-hatched chicks of domestic fowl. DeGennaro (1961) using paper chromatographic technique has shown the glycogen body glycogen to consist of glucose and not other sugars. Glycogen body possesses glycolytic enzymes common to other avian tissues, yet its carbohydrate component appears to be quite stable and resistant to dietary effects. There are controversial views about the effect of starvation on this tissue. Some researchers find no change in the load even when the birds have been starved until death (Doyle

and Watterson, 1949; Szepsenwohl and Michalski, 1951; Watterson, 1958; Hazelwood *et al.*, 1963) while others have opined that stress and starvation altered the glycogen content of this tissue (Graber *et al.*, 1973; Kundu and Bose, 1974). Interestingly the glycogen body lacks the enzyme glucose-6-phosphatase (Benzo *et al.*, 1975; Fink *et al.*, 1975) and hence does not allow this tissue to serve as a nutritive reserve similar to that of liver in avian metabolism. The role of glycogen body in carbohydrate metabolism still remains an enigma. DeGennaro (1959) has shown glycogen body cells growing in culture to accumulate glycogen indicative of synthesis even in absence of neural or hormonal influence. On the other hand, *in vitro* studies of Snedecor *et al.* (1961) and Arond (1968) failed to reveal significant breakdown of glycogen. However, Snedecor *et al.* (1963) later demonstrated incorporation and release of labelled glycosyl units into and from the glycogen of the glycogen body *in vivo*. Benzo and DeGennaro (1974) reported the activities of enzyme phosphorylase and glycogen synthase to be very high in the chick glycogen body. Subsequently, Fink *et al.* (1975) worked out the key enzymes involved in the synthesis and degradation of glycogen *viz.* debrancher, total phosphorylase, brancher, phosphorylase-b-kinase, glycogen synthetase, phosphohexose isomerase, phosphoglucomutase,  $\alpha$  1,4-glucosidase, pyruvate

kinase, phosphofructokinase, fructose 1,6-diphosphatase, fructose-1-phosphate aldolase and UDPG-pyrophosphorylase in the glycogen body of 2-4 week old chicks. Of all the enzymes studied, he found the activities of total phosphorylase, phosphofructokinase and fructose 1,6-diphosphate aldolase to be much higher when compared with normal mammalian liver tissue. In the light of these investigations, DeGennaro (1982) envisages the glycogen body to be metabolically very active since there is evidence of synthesis and breakdown to be continually occurring in the glycogen body.

Fink *et al.* (1975) reported the presence of free glycerol and triglycerides in the glycogen body but at levels substantially lower than those of liver. Earlier work of Friede and Vossler (1964) had failed to demonstrate the presence of lipids in the glycogen body of turkey histochemically.

Among more recent work Syeda <sup>8?</sup> (1989) studied various dehydrogenases and aminotransferase in the avian glycogen body. Of the dehydrogenases studied, the activities of malate, beta-hydroxybutyrate and lactate were very high when compared with different regions of spinal cord. In contrast to this, the activity of succinate was very low,

while isocitrate and glutamate showed moderate activities. Among the aminotransferase, aspartate (AAT) activity was much higher as compared to that of alanine (ALAT).

Administration of mammalian adrenocorticotrophic hormone (ACTH) and pituitary extracts have shown accumulation of glycogen in the glycogen body suggesting some metabolic significance (Hazelwood et al., 1963). Earlier to this, Watterson (1958) had reported a decrease in the glycogen levels following hypophysectomy indicating possible hormonal influence. On the other hand, Thommes and Just (1966) had failed to find any significant difference in the glycogen content of glycogen body between control and hypophysectomized chicks.

Benzo and DeGennaro (1983) have hypothesized that the glycogen stores of this tissue may be metabolically linked to the support of lipid synthesis and myelin formation in the central nervous system during the development of avian embryo. Fink et al. (1975) are of the opinion that glycogen body may serve as a source of organic acids such as pyruvate and lactate to C.N.S. during metabolic assault.

1 does this mean  
energy demand?  
stress  
migration



# ULTRASTRUCTURAL DETAILS:

The first ultrastructural studies on the chick glycogen <sup>were</sup> body have been carried out by Revel and Napolitano (1960) who described glycogen body to contain clear areas of <sup>G</sup>golgi zones associated with many vesicles and low density glycogen granules filling the remainder of the cytoplasm. They observed small aggregation of glycogen within the <sup>G</sup>golgi zone, some being enclosed within the vesicular membrane. Revel et al. (1960) and Revel (1964) found the glycogen particles to consist of  $\beta$ - granules ranging in diameter from 150-600  $\text{\AA}$ . Later Matulionis (1972) working on glycogen body of embryonic chicks (stages 32-44; Hamburger and Hamilton, 1951) recognized three clearly demarkated cytoplasmic areas, a juxtannuclear region with most of the organelles but devoid of glycogen and a peripheral area just below the plasma membrane also free of glycogen and a region flanked by these two with densely packed glycogen granules. He also observed the presence of multivesicular bodies in association with <sup>G</sup>golgi complex with <sup>G</sup>golgi like vesicles in some of the cells. Glycogen body cells showed a paucity of smooth endoplasmic reticulum and an abundance of ribosomes closely associated with glycogen.

Lyser (1973) also studying the ultrastructural morphology of glycogen body, but that of the post-hatched chicks (5-7 days of age) observed the cells to abut onto neural tissue, nerve bundles and ependyma with no special boundary between them. The glycogen body cells containing the blood vessels were adjacent to basal lamina. She found ribosomes in polysomal clusters in the perinuclear area. The glycogen particles of glycogen body appeared similar to glycogen of other astrocytes in chicken (Lyser, 1972) and surmised the glycogen body cells to be specialized neuroglial astrocytes with a capacity to store glycogen in them. Occasionally, glycogen like material was observed in the central canal. However, no glycogen body cell processes were seen crossing the ependyma to enter the central canal as observed by Welsch and Wachtler (1969). Electron microscopic studies of Azcoitia *et al.* (1987) on chicken glycogen body revealed this structure to occupy the arachnoid space. Further they observed presence of glycogen in the subarachnoid space of the lateral and ventral sides of the spinal cord.

DeGennaro and Benzo (1976) based on their electron microscope studies on day old chicks found the glycogen body cells to contain granular endoplasmic reticulum, mitochondria and other cell organelles similar to what was

previously noted by Lyser (1973). Subsequent studies of DeGennaro and Benzo (1991) on the glycogen body of day old Japanese quail, showed the cells to form junctional complex with other adjacent cells, blood vessels, oligodendrocytes, axons, axon terminals etc. He also observed an abundance of smooth endoplasmic reticulum in some of the cells.

#### **FUNCTIONAL ASPECTS:**

That the glycogen body does not serve a functional role in modern birds, but remains a vestigial structure is the view of some workers (Sauer, 1962; Pierce and Fanquy, 1971; Fernandez *et al.*, 1981). These workers surgically removed the glycogen body from adult chicken and found no change in either the behavioural response or to exogeneous endocrines. It is hard to believe that this unusual structure would be without a function, since it houses most of the enzymes concerned with the carbohydrate metabolism. Many researchers (Smith and Geiger, 1961; Welsch and Wachtler, 1969 ;Paul, 1971, 1973) have proposed the glycogen body to be functioning as a supplier of glucose to the C.N.S.. As mentioned earlier, starvation experiments have shown contrasting results with some researchers finding no change in the levels of glycogen

(Doyle and Watterson, 1949; Szepeswohl and Michalski, 1951; Smith and Geiger, 1961; Hazelwood *et al.*, 1963) while other researchers have reported a loss of glycogen under such conditions (Graber *et al.*, 1973; Kundu and Bose, 1974). The proof that some amount of glycogenolysis and glycolysis occurs in the glycogen body comes from the work of Snedecor *et al.* (1963) who demonstrated incorporation and release of labelled glycosyl units within the glycogen of the glycogen body *in vivo*. The reason behind the non-varying polysaccharide may be a total lack of glucose-6-phosphatase (Benzo *et al.*, 1975 and Fink *et al.*, 1975). According to DeGennaro (1982), the glucose formed may not be released or lost from the tissue but may be getting committed to other metabolic pathways including resynthesis of glycogen.

Benzo and DeGennaro (1983) are of the opinion that glycogen body may be functioning as a generator of NADPH which in turn may be primarily directed to lipogenesis. They found high activities of enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the glycogen body when compared with liver and muscles. A possible function of glycogen body could be supporting myelin synthesis in the developing nervous system. Another could conceivably be to maintain the complement of fatty

acids for the cerebrospinal fluid (DeGennaro, 1974; DeGennaro and Benzo, 1978; Benzo and DeGennaro, 1983).

Syeda (1989) does not fully agree with the idea that NADPH produced will be utilized for the purpose of fatty acid synthesis, since NADPH is required for a number of biochemical reactions such as (1) separation of the two sulfur atoms of the disulphide bond between the two oxidized glutathione molecules catalyzed by glutathione reductase, (2) reduction of glucuronic acid to L-gulonic acid, (3) reductive carboxylation of pyruvic acid to malic acid by malic enzyme, (4) reduction of dihydrofolic to tetrahydrofolic acid etc. According to Syeda (1989), high activities of the hexose monophosphate shunt enzymes may be for the purpose of supplying ribose sugars needed for the formation of nucleosides, especially uridine. The main accent of this tissue being on glycogen synthesis large quantities of UTP would be required to form UDPG. As mentioned above the shunt would also provide NADPH to keep glutathione in reduced state. Syeda (1989) observed high activity of enzyme glutamine synthetase (GS) and moderate activity of glutamate dehydrogenase (GDH) in the glycogen body of developing chicks. Moderate GDH (although low GS) was also found in glycogen body of adult blue rock pigeon. Since bird flight involves a high neuromuscular activity

which is known to generate ammonia, glycogen body was seen as a detoxifying appendage of avian C.N.S..

E.M studies of DeGennaro (1982) have showed the glycogen body cells to be in contact with each other forming an intercellular canalicular system and based on this findings Azcoitia *et al.* (1987) proposed the glycogen body cells to 'secrete' the subarachnoid glycogen like any astrocyte (Lumsden, 1968) they surmise that " Any elevation in the osmotic pressure of the cerebrospinal fluid caused by the presence of glycogen would draw this fluid from the nervous parenchyma into the meningeal space ".

Moving from glycogen body to spinal cord of birds. it is seen that the cord extends throughout the whole length of the spinal (vertebral) canal, but unlike mammals does not have either a cauda equina nor a pronounced filum terminale (Benzo , 1986). As in the case of other vertebrates, the spinal cord of birds also have two prominent enlargements *viz.* cervical and lumbosacral, but the latter is considerably larger compared with the other vertebrates. Since the activities of the limb musculature are mainly controlled by these regions, the gray matter here is more massive. The axonal network which form the

brachial and lumbosacral plexi that supply the limbs (Brinkman and Martin, 1973; Martin, 1979) originate from these areas.

Earlier work from this laboratory (Syeda, 1989) has shown the avian spinal cord regions having more of gray matter areas to exhibit greater activities of most enzymes namely, glutamate dehydrogenase, glutamine synthetase and lactate, succinate, malate and butyrate dehydrogenases and also of aspartate amino-transferase. In continuation of the earlier work, an attempt has been made in the present study to include data on the different regions of spinal cord wherever possible. It was decided to extend the studies by looking at the electrophoretic pattern of lactate dehydrogenase in the four regions of spinal cord viz. cervical, anterior thoracic, posterior thoracic and lumbosacral in the post-hatched developing chicks of domestic fowl and adult pigeon. Cervical region of developing fowl and adult pigeon was reported by Syeda (1989) to have more activity of enzyme glutamine synthetase. Moreover, it is rich in nerve tracts but comparatively poor in neurons whereas the lumbosacral cord is richer in neurons but poorer in nerve tracts. This prompted the work on free amino acids in the cervical and lumbosacral spinal cord of 2 week old post-hatched chick.



### SCOPE OF PRESENT WORK:

Survey of literature thus reveals several glaring lacunae and leads to numerous queries. They are as follows:

- (1) Can the values of glycogen body glycogen as reported by Syeda (1989) be so high? Secondly<sup>(2)</sup>, do the glycogen values show a significant drop during night hours of summer months but not during winter months? The experimental protocol adopted by the worker was obtained (personal communication) and analysed critically. On doing this certain flaws were noticed, the main being unavailability of data on successive years. It was decided to repeat the earlier work making necessary modifications. This reevaluation forms the subject matter of Chapter 1.

Glycogen body shows a very high glycolytic activity. Dezza *et al.* (1970) reported very high levels of lactate and pyruvate in the glycogen body of 6-8 week old chicken. The values were comparable to those produced by liver slices incubated under similar conditions. Moreover, glycogen body also possesses high activity of enzyme lactate dehydrogenase in turkey (Friede and Vossler, 1964) and the chicken (Syeda, 1989). In the light of above findings, it <sup>was</sup> has been decided to investigate the temporal changes in the isoenzyme profile of lactate

dehydrogenase not only in the glycogen body but also in four regions of spinal cord of neonatal chicks of domestic fowl (1-50 days of age). Pattern in the adult birds was also looked into for comparison (Chapter 2).

If astroglial cells of glycogen body like astroglial cells in C.N.S. were to participate in the detoxification of ammonia this should be reflected in its amino acid profile. Chapter 3 includes thin layer chromatographic study on amino acids in the glycogen body of the two week post-hatched chicks. For comparison two regions of spinal cord, one rich in nerve tracts but numerically poor in neurons and the other rich in neurons but poorer in number of nerve tracts were also studied.

Finding chromatographically high levels of glutamate in glycogen body was supportive of its possible role in detoxification of ammonia. Moreover, moderate levels of GDH and high levels of GS were reported by Syeda (1989) in developing chicks. It was decided to assess the detoxifying potential by exposing the 5 day old chicks to high doses of ammonia. Since astrocytes are known to accumulate glycogen and show deranged structure in hepatic encephalopathy caused by ammonia toxicity (Norenberg, 1981), it was decided to study the effect of ammonia on

this parameter, namely, glycogen and also on glycogen synthesizing and degrading enzymes. The details are described in chapter 4.

If the glycogen body were involved in the synthesis of lipids required for the formation of myelin during the development of avian nerve cord as proposed by DeGennaro (1982) then why should this tissue persist in adult bird? *Regenerative, maintenance*  
 So far no one has taken yet another possibility into account, namely, its role in extracellular matrix formation, especially during early development. Since labelled glycosyl units are known to be added to as well as removed from glycogen of glycogen body (Snedecor et al., 1963) it is possible that G-1-P released by phosphorylase action may be converted into any of the uronic acids, either glucuronic or galacturonic or any of its derivatives such as hyaluronic acid. It can also get converted into any of the amino sugars or N-acetylated amino sugars. Both uronic acid (including their derivatives) and amino sugars (and their derivatives) are constituents of glycoproteins. This possibility is discussed in Chapter 5 which covers E.M work on adult pigeon. Earlier E.M studies of the chicken glycogen body revealed presence of <sup>G</sup>golgi with dense vesicles (Revel et

et al., 1960; 1964). Present work is an effort to study further details especially of <sup>G</sup>golgi vesicles and perinuclear cytoplasm.

Like ammonia, fluoride is another toxic agent that affects the C.N.S.. Fluoride is known to cross blood-brain barrier. Since literature on the effects of fluoride on astrocytes could not be traced, it became imperative to work out the effect of sodium fluoride on some of the metabolic parameters in the glycogen body of post-hatched developing chicks (1-30 days of age). The same is detailed in <sup>G</sup>chapter 6.